# ab302933 – Aflatoxin B1(AFB1) ELISA Kit

For the in vitro quantitative determination of Aflatoxin B1 concentrations in feedstuff, feed, grain, edible oil.
For research use only - not intended for diagnostic use.

**For overview, typical data and additional information please visit:**
https://www.abcam.com/ab302933

## Storage and Stability

On receipt entire assay kit should be stored at 4°C for up to 12 months from the date of shipment. Upon opening, use kit within 1 month stored at 4°C. Do not use the kit beyond the expiration date.

## Materials Supplied

| Item | Quantity | Storage Condition |
|---|---|---|
| Assay Plate | 8 X 12 Strips | 4°C |
| Standard | 5 x 1 mL | 4°C |
| HRP-conjugate | 1 x 7 mL | 4°C |
| Antibody | 1 x 7 mL | 4°C |
| TMB Substrate | 1 x 12 mL | 4°C |
| Stop Solution | 1 x 10 mL | 4°C |
| Sample Diluent | 1 x 50 mL | 4°C |
| Wash Buffer (10×) | 1 x 50 mL | 4°C |
| Adhesive strip | 4 | 4°C |

## Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Chemicals: Methanol, Ethanol, NaOH
- Microplate reader capable of measuring absorbance at 450 nm
- An incubator that provides stable incubation condition at 25°C
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Centrifuge, vortex
- Analytical balance, 2 decimal places
- Single-channel micropipettes (20-200 µL,100-1000 µL,1000-5000 µL)
- 300 µL multichannel pipette
- Precision pipettes with disposable tips
- Distilled or deionized water

## PRECAUTION

The Stop Solution is acidic. Wear eyes, hands, face, and clothing protection when using the product.

**Δ Note:**

- Kindly use graduated containers to prepare the reagent.
- Bring all reagents to room temperature (20-25°C) before use for 30 min.
- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

## Reagent Preparation

- Bring all reagents to room temperature (20-25°C) 30 minutes before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

**Wash Buffer (1x):** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 10 mL of Wash Buffer (10x) into 90 mL deionized or distilled water to prepare 100 mL of Wash Buffer (1x). Keep it at 4 °C for one month.

**70% Ethanol:** Transfer 700 mL of Ethanol and mix it with 300 mL of deionized or distilled water. Shake well.

**40% Ethanol:** Transfer 400 mL of Ethanol and mix it with 600 mL of deionized or distilled water. Shake well.

**Δ Note**

- Abcam is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their experiments.

## Standards Concentration:

| Standards | S1 | S2 | S3 | S4 | S5 |
|---|---|---|---|---|---|
| Concentration (µg/kg) | 0 | 0.15 | 0.45 | 1.35 | 4.05 |

## Sample Preparation and collection

**Δ Note:** The prepared sample may be stored for up to one day at 2-8°C.

**Edible oil:** Take 5.00 ± 0.05 g of edible oil and add 25 mL of **70% Ethanol**, vortex well for 2 min. Centrifuge at 4000 rpm for 5 minutes. Transfer 100 µL of sample supernatant and transfer it to the new centrifugal tube. Add 100 µL of Sample Diluent and mix well. Take 50 µL of sample for further analysis. Dilution factor of sample: 10.

**Feedstuff, feed, grain:** Weigh 5.00 ± 0.05 g of the homogenized sample. Add 25 mL of 40% Ethanol and vortex for 5 minutes. Centrifuge sample at 4000 rpm for 5 minutes. Transfer 200 µL of supernatant into a new centrifugal tube, add 600 µL of **Sample Diluent**, shake well. Take 50 µL of sample for further analysis. Dilution factor of sample: 20. (Adjust the pH to 6-7 by adding NaOH when the pH < 6).

## Assay Protocol

- Bring all reagents and samples to room temperature 20-25 °C, centrifuge them after thawing before commencing with assay.
- It is recommended that all standards and samples be run at least in duplicate.

1. Prepare all reagents and samples as instructed in reagent and sample preparation section above.
2. Determine the number of wells to be used and put any remaining assay plates and other reagents back into the pouch and seal the Ziploc; store unused wells at 4°C.
3. Add 50 µL of **Standard** or **Sample** per well. Follow by adding 50 µL of both **HRP-conjugate** and 50 µL of **Antibody** to each well. Cover the assay plate with a new adhesive strip and mix well, then incubate for 15 min at 25°C.
4. Aspirate each well and wash, repeating the process 4 times. Wash by filling each well with 250 µL of **Wash Buffer (1X)** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds. Complete removal of liquid at each step is essential to good performance.
5. Add 100 µl of **TMB Substrate** to each well, mix well. Incubate for 5 minutes at 25°C. Protect from light.
6. Add 50 µl of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
7. Determine optical density (OD) result at 450 nm within 5 minutes. (Recommend reading the OD value at the dual-wavelength: 450/630 nm).

## Calculation of Results

**Δ Note:** The OD value of the sample has a negative correlation with Aflatoxin B1 in the sample.
For example:

| Sample | Concentration (µg/kg) | OD Standard |
|---|---|---|
| **1** | 0 | 1.926 |
| **2** | 0.15 | 1.679 |
| **3** | 0.45 | 1.248 |
| **4** | 1.35 | 0.711 |
| **5** | 4.05 | 0.259 |

Then the Aflatoxin B1 for an OD of 0.653 is 1.35 - 4.05 µg/kg and for an OD of 1.419 it would be 0.15 - 0.45 µg/kg.
Lastly the reader is multiplied by the corresponding dilution factor of each sample and the actual concentration of sample is obtained.

**Δ Note:**

- Discard the substrate with any color that indicates the degeneration of this solution. When the absorbance value of S1 less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25 °C and too high or too low will result in the changes in the absorbance value and detecting sensitivity.

**Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:**
www.abcam.com/protocols/the-complete-elisa-guide

## Technical Support


For all technical or commercial enquiries please go to:

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